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Two Methyltransferase Activities in the Purified Virions of Vesicular Stomatitis Virus

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In addition to an RNA-dependent RNA polymerase, purified vesicular stomatitis virus contains a methyltransferase activity which transfers the methyl group from the methyl donor, *S*-adenosyl-L-methionine, to two positions in the 5'-terminal capped structure of the nascent mRNA's synthesized in vitro as ^{7}mG(5')ppp(5')A$_{p}^{m}$.... In the present study it is shown that two distinct methyltransferase activities are discernible in the purified virus. The in vitro concentrations of the methyl donor specify the number and location of the methyl groups transferred to the capped 5'-termini of VSV mRNA's. Limited concentrations of the methyl donor result in a single methylation of the penultimate base in the 2'-hydroxyl position, that is, G(5')ppp(5')A$_{p}^{m}$..., whereas saturating concentrations of the methyl donor methylate the blocking guanosine residue at the 7-position, resulting in the dimethylated cap, ^{7}mG(5')ppp(5')A$_{p}^{m}$.... Pulse-chase experiments demonstrate that the monomethylated cap structure is the precursor substrate for the dimethylated cap. In this respect, vesicular stomatitis virus system is quite distinct from the vaccinia and reovirus systems. Virus purified from different host cells including hamster, mouse, and human contain both methyltransferase activities. The mRNA's containing monomethylated capped structures are poor templates for protein synthesis in vitro.

Purified virions of vesicular stomatitis virus (VSV) contain an RNA-dependent RNA polymerase (5) which transcribes the negative strand genome RNA in vitro into five monocistronic RNA species (24). Recent experiments indicate that the polymerase initiates transcription at the 3'-terminus of the genome (12) with the synthesis of a short leader RNA sequence followed by the sequential synthesis of the VSV mRNA species (2, 4). The 5'-terminus of the leader RNA is unblocked and polyphosphorylated with the sequence (p)ppA-C-G (11), in contrast to all the mRNA species which contain a blocked 5'-terminal structure with the sequence G(5')ppp(5')A-A-C-A-G (27). In the presence of the methyl donor *S*-adenosyl-L-methionine (SAM), a methyltransferase activity is discernible in the purified virion which methylates the 5'-terminal blocked structure of the mRNA's as ^{7}mG(5')ppp(5')A^{m}... (3). The 5'-terminus of the leader RNA is neither blocked nor methylated under these conditions.

Similar blocking and methyltransferase activities have also been found to be present in the purified virions of reo (17), vaccinia (29), cytoplasmic polyhedrosis (16), Newcastle disease (13), and wound tumor viruses (28). In the vaccinia virus system, two methyltransferase activities have been solubilized (21, 22): one which methylates the blocking guanosine residue at the 7 position only, and the other which uses this methylated substrate to methylate the penultimate base at the 2'-hydroxyl position (15; Barbosa and Moss, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, S186, p. 310). A methyltransferase activity, which can methylate only the 2'-hydroxyl group of the penultimate base, has not been reported. Thus, the 5'-terminal structure of the form G(5')ppp(5')X$_{p}^{m}$... was not detected in any of the virus systems mentioned above. The blocking and methylation processes in VSV appear to be transcription dependent, since methods similar to those employed with vaccinia virions have failed to solubilize these activities (1).

In this communication, we report that two distinct methyltransferase activities can be detected in the purified virions of VSV. At low concentrations of SAM, the methylation occurs exclusively in the penultimate adenosine base of the 5'-termini of the VSV mRNA's as G(5')ppp(5')A$_{p}^{m}$.... As the concentration of SAM is increased, a second methyltransferase activity is observed which catalyzes the methylation of the guanosine residue of the capped structure as ^{7}mG(5')ppp(5')A$_{p}^{m}$....

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MATERIALS AND METHODS

Purification of VSV. VSV (Indiana serotype) was grown in baby hamster kidney cells (BHK-21, clone 13 adapted to suspension culture), L cells, and HeLa cells and purified as described previously (6).

Synthesis and purification of RNA in vitro. RNA was synthesized in vitro in the standard incubation mixture as described previously (7) except when labeling with α-[^{32}P]GTP or α-[^{32}P]UTP, when the concentration of GTP or UTP was lowered to 100 μM. Incubation was at 30°C for 3 h. The reaction was terminated by the addition of sodium dodecyl sulfate to 0.5%, and the product RNA was extracted with phenol. The poly(A)-containing mRNA's were purified by binding and subsequent elution from oligo(dT)-cellulose columns (Collaborative Research) as described previously (7). The polyadenylated mRNA's were pooled after oligo(dT)-cellulose chromatography and precipitated with ethanol prior to analysis.

5'-Terminal structure analysis of VSV mRNA. Procedures used for the enzymatic digestion of RNA, paper electrophoresis, and paper chromatography have been described in detail (24).

Translation of VSV mRNA in cell-free extracts of wheat germ. The preparation of wheat germ extracts and the conditions used for protein synthesis have been described (8).

Unmethylated or methylated poly(A)-containing VSV mRNA's (0.2 to 0.25 μg) were incubated with wheat germ extract at 25°C in the presence of 200 μM Anisomycin to prevent polypeptide elongation. *S*-adenosylhomocysteine (SAH) (400 μM) was included to prevent methylation by enzymes present in the cell-free extract.

Radioisotopes. *methyl*-^{3}H-labeled *S*-adenosyl-L-methionine (specific activities, 7.5, 9.7, and 67.9 Ci/mmol), α-[^{32}P]UTP (specific activity, 13.7 Ci/mmol), and α-[^{32}P]GTP (specific activity, 10.1 Ci/mmol) were purchased from New England Nuclear, Boston, Mass. L-[^{35}S]methionine (specific activity, 323 Ci/mmol) was purchased from Amersham-Searle.

RESULTS

Mono- and dimethylated, blocked 5'-termini of VSV mRNA's. In our previous studies (3) methylation of VSV mRNA's in vitro was carried out in the presence of saturating amounts of SAM (>7 μM). At this concentration, two methyl groups were incorporated into the 5'-terminal blocked structure of VSV mRNA in the form ^{7}mG(5')ppp(5')A$_{p}^{m}$Ap.... It was of interest to determine whether the two observed methyltransferase activities are manifested by a single enzyme or, in fact, by two separate enzymes.

VSV mRNA's were synthesized in vitro in the presence of different concentrations of ^{3}H-labeled SAM to determine the extent of methylation. The RNA species synthesized in the presence of different concentrations of SAM were identical to the species synthesized in the absence of SAM (data not shown). The poly(A)-containing mRNA's were purified, and the 5'-terminal blocked structures were analyzed by high-voltage paper electrophoresis after the appropriate enzyme treatment of mRNA's. A typical electropherogram of the blocked structures formed in the presence of 2.2 μM SAM is shown in Fig. 1A. In contrast to our previous observation, two distinct methylated and blocked 5'-terminal structures were found: one migrating between the markers pA and pG (designated as Cap A) and the other migrating ahead of pG (designated as Cap B). The Caps A and B were eluted from the paper and further analyzed after

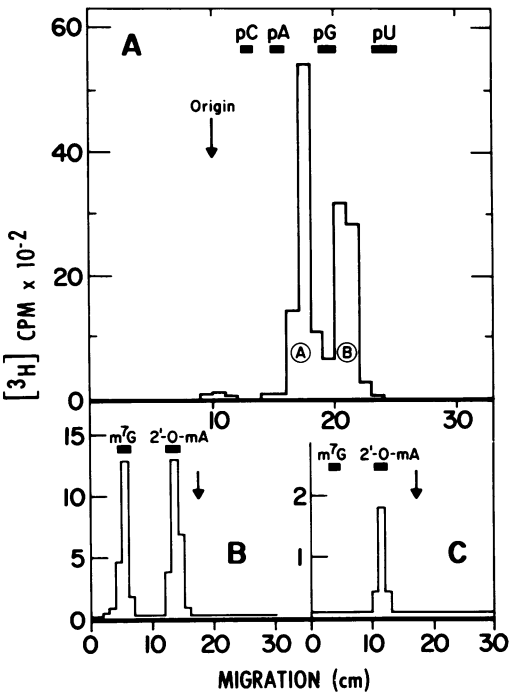

FIG. 1. *Paper electrophoretic analyses of enzymatic digestions of methyl-^{3}H-labeled VSV mRNA synthesized in vitro. VSV mRNA was synthesized in vitro in the presence of ^{3}H-labeled SAM (2.2 μM) as the labeled substrate. The purified mRNA was digested with penicillium nuclease followed by bacterial alkaline phosphatase and analyzed directly by paper electrophoresis (A). After electrophoresis, the radioactivity was eluted from the paper, further digested by nucleotide pyrophosphatase followed by bacterial alkaline phosphatase, and reanalyzed by electrophoresis (B and C). Marker nucleotides and nucleosides were included in each sample, and their positions after electrophoresis are shown. (B) The material from panel A designated as region A. (C) The material from panel A designated as region B.*

digestion with nucleotide pyrophosphatase and bacterial alkaline phosphatase. As shown in Fig. 1B, the Cap A was resolved into two ^{3}H-labeled nucleosides which migrated towards the cathode with the markers ^{7}mG and 2'-*O*-mA. The Cap B, on the other hand, produced a single ^{3}H-labeled nucleoside which comigrated with 2'-*O*-mA (Fig. 1C). These results clearly indicate that the two methylated 5'-terminal blocked structures present in VSV mRNA's at this SAM concentration can be designated as ^{7}mG(5')ppp(5')A^m (Cap A) and G(5')ppp(5')A^m (Cap B). Thus, it seems that a separate 2'-*O*-methyltransferase activity in addition to 7-methylguanosine methyltransferase is present in purified VSV.

To confirm that the Cap B structure was indeed of the form G(5')ppp(5')A^m and not A(5')ppp(5')A^m, or A^m(5')ppp(5')A^m, VSV mRNA's were synthesized in the presence of ^{3}H-labeled SAM and α-[^{32}P]GTP as the labeled RNA precursors. The purified, labeled RNA was treated with nuclease P$_1$ and bacterial alkaline phosphatase, and the reaction products were analyzed by high-voltage paper electrophoresis. The presumptive cap structure contained both ^{32}P and ^{3}H radioactivity and migrated with the marker pG (Fig. 2A). The ^{32}P$_i$ released during the reaction migrated beyond 30 cm and is not shown in the figure. The radioactivity in Fig. 2A was eluted and treated with nucleotide pyrophosphatase and analyzed similarly. As shown in Fig. 2B, all the ^{32}P migrated with pG and the ^{3}H migrated with pA, consistent with results that the cap structure was of the type G$\overset{*}{p}$ppA^m or Cap B. The ^{3}H radioactivity migrating with pA in Fig. 2B was confirmed to be 2'-*O*-mA by paper chromatography with authentic marker (Fig. 2C).

We next determined the distribution of Cap A and Cap B in VSV mRNA's as a function of SAM concentration. As shown in Fig. 3, at a low concentration of SAM (<0.1 μM), the predominant methylated blocked 5'-termini is of Cap B form (>95%), whereas with higher concentrations of SAM (>7 μM) the Cap A form constitutes more than 80% (see also Fig. 6). The reason for not detecting the Cap B form at higher concentrations of SAM in our previous studies is primarily due to unavailability of commercial ^{3}H-labeled SAM at sufficiently high specific activity. The above results indicate that, at low concentrations of SAM, only the 2'-*O*-methyltransferase activity in VSV can be detected; with increasing concentration of SAM, the 7-methylguanosine methyltransferase activity appeared. The above data are consistent with the notion that the Cap B structure is the precursor for the Cap A structure. This observation is also consistent with the inability to find mono-

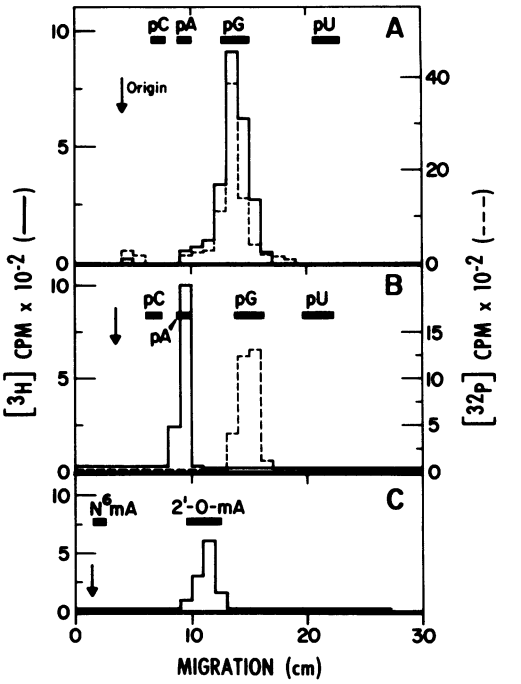

FIG. 2. *Paper electrophoretic and chromatographic analyses of enzymatically digested methyl-^{3}H-labeled VSV mRNA also labeled with α-^{32}P-GTP. The poly(A)-containing VSV mRNA synthesized in vitro in the presence of α-^{32}P-GTP (specific activity 2,750 cpm/pmol) and ^{3}H-labeled SAM (0.037 μM) was purified and digested with penicillium nuclease followed by bacterial alkaline phosphatase prior to electrophoretic analysis (A). After electrophoresis, the radioactivity was eluted from the paper and digested with nucleotide pyrophosphatase prior to reanalysis by electrophoresis (B). The methyl-^{3}H radioactivity migrating with the pA marker was eluted and analyzed by ascending chromatography on Whatman DE-81 paper presoaked in 1% boric acid prior to chromatography with water as the solvent (C). The positions of marker nucleotides and nucleosides were included in each sample as indicated.*

methylated cap structures of the form ^{7}mG(5')ppp(5')A$_p$ ... in the RNA products under low (Fig. 2) and high (3) concentrations of SAM. It should be further noted that, based on the extent of incorporation of methyl groups and RNA synthesis at SAM concentrations of 0.1 μM and 10 μM, the number of methyl groups per RNA molecule was found to be unity and two, respectively, also indicating that the ribose methylation precedes 7-methylation of the guanosine base.

Effect of SAH on the methylase activities. From the above results, it was apparent that the 2'-*O*-methyltransferase activity possessed a considerably lower K_m than the 7-methyltrans-

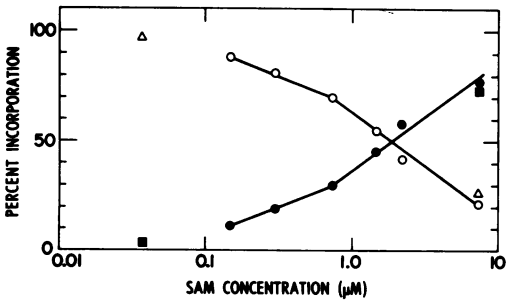

FIG. 3. *Distribution of methyl-^{3}H-labeled 5'-termini in Caps A and B as a function of the SAM concentration. VSV mRNA was synthesized in vitro in the presence of increasing amounts of ^{3}H-labeled SAM (67.9 Ci/mmol) as the labeled substrate. The purified mRNA was enzymatically digested with penicillium nuclease followed by bacterial alkaline phosphatase prior to electrophoretic analyses as shown in Fig. 1. The relative proportion of methyl-^{3}H radioactivity in Cap A (●, ■) and Cap B (○, △) (where Cap A + Cap B = 100%) is presented for each SAM concentration used. The symbols △ and ■ represent separate experiments.*

ferase activity. From double-reciprocal plots of the kinetics of methylation and the SAM concentrations, the apparent K_m of the former activity was calculated as 0.5 μM and the corresponding value of the latter activity was 10 μM (data not shown). Thus, 7-methylguanosine methyltransferase activity requires approximately 20-fold higher concentration of SAM than the corresponding 2'-*O*-methyltransferase activity.

We next determined the effect of SAH, a competitive inhibitor of methyltransferases, on the two methyltransferase activities. As expected, the 7-methylguanosine transferase activity, which demonstrated a higher K_m, was inhibited first (>90%) at an SAH-to-SAM ratio of 1, whereas at the same concentration of the inhibitor the 2'-*O*-methyltransferase activity was only inhibited by 60% (Fig. 4). These experiments show that two distinct methyltransferase activities are present in purified VSV.

Conversion of Cap B to Cap A. The relative distribution of Caps A and B (Fig. 3) was suggestive that Cap B is the precursor of Cap A. To directly test this possibility, a pulse-chase experiment was performed. mRNA's containing Cap B were synthesized in vitro at a low SAM concentration. After 20 min, the fate of the pulsed label was determined by a chase with 2,700-fold excess of unlabeled SAM (Fig. 5). Under the latter condition, only the Cap A type structure was formed. The analysis of the Cap A structure after the chase indicated that Cap B was indeed the precursor of Cap A and as much as 30% of Cap B can be chased into the Cap A structure over a period of 30 min.

Methyltransferase activities in VSV purified from different host cells. Since two distinct methyltransferase activities were found in purified VSV, it was of interest to determine whether similar activities were also discernible

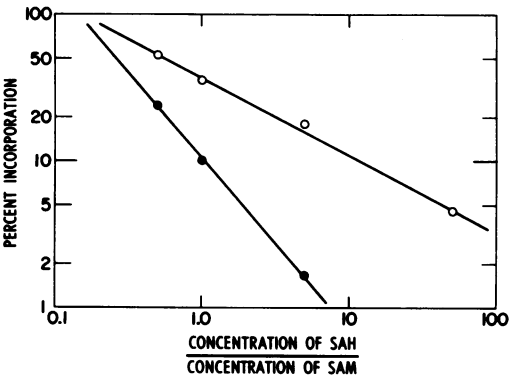

FIG. 4. *Differential inhibition of methylation of Caps A and B by SAH. VSV mRNA was synthesized in vitro in the presence of ^{3}H-labeled SAM (10 μM) as the labeled substrate and increasing quantities of SAH. The purified RNA was digested with penicillium nuclease followed by bacterial alkaline phosphatase prior to electrophoretic analyses. The proportion of methyl-^{3}H radioactivity in Cap A (●) and Cap B (○) is presented for each concentration of SAH used. (The initial counts per minute used for RNA containing Cap A is approximately 2,000).*

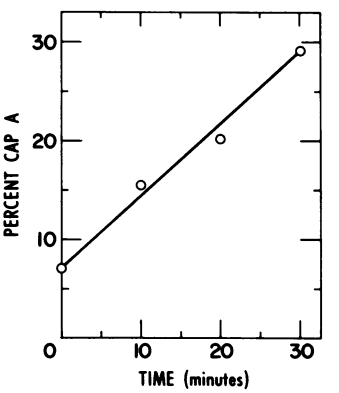

FIG. 5. *Kinetics of Cap A synthesis. VSV mRNA was synthesized in vitro at 25°C in the presence of ^{3}H-labeled SAM (0.037 μM) as the labeled substrate. After a 20-min preincubation period, unlabeled SAM was used to adjust the concentration to 100 μM, and aliquots of the reaction mixture were removed. The mRNA was purified and analyzed by paper electrophoresis after enzymatic digestion by penicillium nuclease followed by bacterial alkaline phosphatase. The relative proportion of methyl-^{3}H radioactivity in Cap A is presented as a function of time.*

in VSV purified from different host cells. VSV was purified from HeLa and L cells, and the methyltransferase activities of the virions were compared with the virions purified from BHK cells. As shown in Fig. 6, the two methyltransferase activities responsible for the syntheses of both Cap A and Cap B were also present in the virions purified from HeLa and L cells. These results suggest either that both of the methyltransferase activities could be virus-specific proteins or, if cellular components, that they are present in at least three different cell lines including hamster, mouse, and human.

Translational efficiency of Cap B-containing mRNA's. It has been well documented that, in cell-free protein-synthesizing extracts derived from wheat germ (8, 25), or *Artemia salina* embryos, translational efficiences are considerably greater for the fully methylated forms of reovirus and VSV mRNA's than their unmethylated counterparts. Although the presence of ^{7}mG in the capped 5'-terminal structure of mRNA's seemed to play an important positive role in protein synthesis and ribosome binding, the precise role of 2'-*O*-methylation of the 5'-penultimate base in a similar situation is presently unclear. The reason for this is due to the unavailability of eukaryotic mRNA's, reported so far, to contain the 5'-cap structure of the form G(5')ppp(5')X_p^m ... (X = purine or pyrimidine). The VSV mRNA's containing a 5'-terminal Cap B structure, i.e., G(5')ppp(5')A_p^m ..., offer an opportunity to study the role, if any of 2'-*O*-methylation in protein synthesis. VSV polyadenylated mRNA's containing 5'-terminal unmethylated, monomethylated, and dimethylated capped structures were translated in vitro in a wheat germ protein-synthesizing system. From the results shown in Fig. 7, the following conclusions can be drawn. (i) mRNA's containing the 5'-dimethylated capped structure, i.e., Cap A, are efficiently translated in the wheat germ system as observed previously (8). (ii) The unmethylated and Cap B-containing mRNA's are not translated and demonstrate values that are similar to the control experiments containing no added RNA. (iii) In the above two experiments, the translation was carried out in the presence of a large excess of SAH to prevent methylation by endogenous methylase(s) in wheat germ extracts. But when Cap B-containing mRNA's were translated in the absence of SAH, a slight but distinct incorporation of ^{35}S into protein was observed, suggesting that the conversion of Cap B to Cap A by the endogenous methylases is required for efficient translation (8). (iv) When the translation was carried out in the presence of 1 mM ^{7}mGMP, all mRNA's including the Cap A-containing ones were totally inhibited indicating that there is a structural requirement of ^{7}mG in mRNA's which facilitates their binding to ribosomes and translation (19). These results indicate that, in the wheat germ extracts, the Cap B-containing mRNA's failed to initiate protein synthesis indicating that methylation of the blocking guanosine residue plays an important role in the process of initiation, whereas 2'-*O*-methylation of 5'-penultimate base presumably is inert in this process.

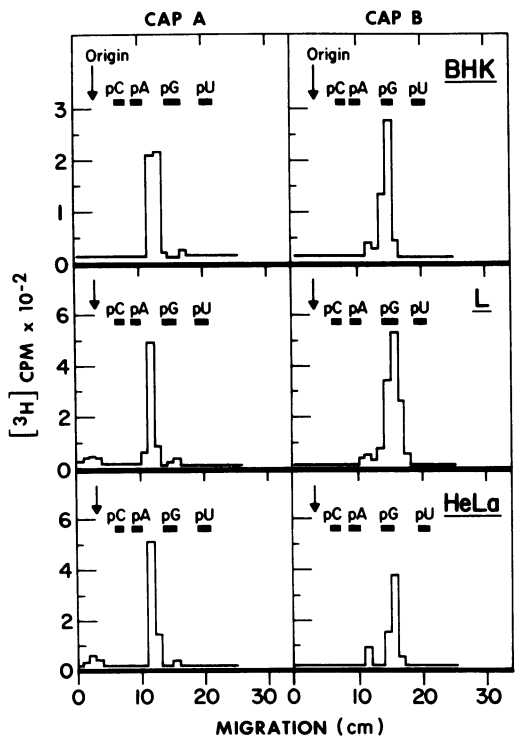

FIG. 6. *Paper electrophoretic analyses of Caps A and B synthesized in vitro by VSV purified from different cell lines. VSV was purified from BHK, L, and HeLa cells, and mRNA was synthesized in vitro in the presence of ^{3}H-labeled SAM at final concentrations of 13.3 μM (Cap A) or 0.037 μM (Cap B). The purified mRNA was digested with penicillium nuclease followed by bacterial alkaline phosphatase and analyzed by paper electrophoresis with marker nucleotides.*

DISCUSSION

Previously it has been shown that the purified virions of VSV contain a methyltransferase activity which in vitro catalyzes the incorporation of methyl groups from the methyl donor to the 5'-terminal blocked structure of VSV mRNA's as ^{7}mG(5')ppp(5')A_p^m.... Similar activities have also been reported in other animal viruses which include reo (17), vaccinia (29), cytoplasmic polyhedrosis (16), and wound tumor viruses (28).

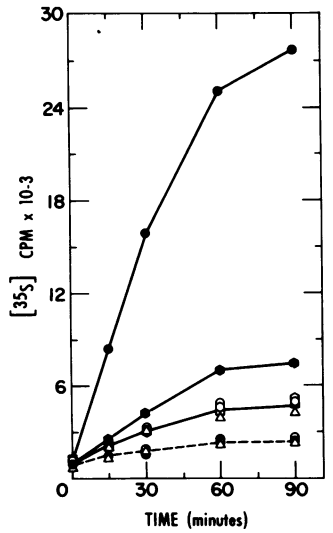

FIG. 7. *Role of ribose methylation on protein synthesis in vitro. In vitro synthesized, methylated, and unmethylated polyadenylated VSV mRNA's were used to stimulate protein synthesis in cell-free extracts of wheat germ (8). VSV RNA was synthesized in vitro in the presence of [α-32P]-UTP and in the absence or presence of 0.037 μM (low) and 13.3 μM (high) 3H-labeled SAM concentrations. The RNA's were purified as described in the text. The integrity of the mRNA species were checked by velocity sedimentation (3). Endogenous methylation of the VSV mRNA's by the wheat germ extract was prevented by the addition of SAH (400 μM) to the 50-μl translation reaction mixture. Protein synthesis was assayed as [35S]methionine incorporation into acid-precipitable material directed by 0.25 μg of VSV mRNA in the presence (broken line) or absence (solid line) of 7mGMP (1 mM) as a competitive inhibitor of mRNA ribosome binding. Symbols: Cap A (●); Cap B (○); unmethylated VSV mRNA synthesized in vitro in the presence of 750 μM SAH (◇); or in the absence of SAM (△); no added RNA (□). The symbol ● represents the activity of Cap B mRNA incubated in a wheat germ extract in the absence of SAH.*

The notable exception is Newcastle disease virus which contains only the 7-methylguanosine methyltransferase activity (13). Considerable work has been done with the virion-associated methyltransferases of vaccinia virus. Two enzyme activities have been detected in these purified virus cores (21, 22). The mRNA (guanosine-7-) methyltransferase activity (molecular weight 127,000) which has been purified transfers a methyl group from SAM to the 7-position of the blocking guanosine residue. The second activity, although not purified, methylates the 2'-hydroxyl moiety of the 5'-base presumably by using the blocked and methylated mRNA's of the form ^{7}mG(5')ppp(5')Np ... as the substrate. Moreover, it has been shown that, at limited concentrations of SAM, partially methylated 5'-terminal structures of form ^{7}mG(5')ppp(5')-Np ... were obtained. Thus, in the vaccinia system the methylation at the 7-position of guanosine is the first methylation event followed by the ribose methylation of the penultimate base (23). A similar methylation sequence has also been shown in the reovirus system (18).

Results presented in this communication clearly show that the methylase and the methylation reactions in the VSV systems are quite different from those observed with vaccinia and reoviruses. In the VSV system, two distinct methyltransferases can be detected. At limiting concentrations of SAM, the 5'-terminal blocked structure of the mRNA's synthesized in vitro is of the form G(5')ppp(5')A_p^m ... (Cap B) (Fig. 1 and 2). With increasing concentrations of SAM, the guanosine-7-methyltransferase activity is detected and the dimethylated 5'-terminal structure of VSV mRNA's of the form ^{7}mG(5')-ppp(5')A_p^m... (Cap A) is obtained. Moreover, by pulse-chase experiments it can be shown that the mRNA's containing Cap B structure are the precursor of the eventual conversion into Cap A-containing mRNA's (Fig. 6). Thus, in the VSV system the sequence of events leading to the fully methylated 5'-terminal structure of VSV mRNA's in vitro can be depicted as:

$$\text{nGTP} + \text{nATP} + \text{nUTP} + \text{nCTP} \rightarrow \text{G(5')ppp(5')ApApCpApGp} \ldots + \text{nPP}_i$$

$$\text{G(5')ppp(5')Ap} \ldots + \text{SAM} \rightarrow \text{G(5')ppp(5')A}_p^m \ldots + \text{SAH}$$

$$\text{G(5')ppp(5')A}_p^m \ldots + \text{SAM} \rightarrow {}^7\text{mG(5')ppp(5')A}_p^m \ldots + \text{SAH}$$

This sequence of methylation is different from the vaccinia and reovirus systems, where 7-methylation of guanosine precedes 2'-*O*-methylation of the penultimate base (18, 23).

The two methylase activities associated with VSV have different affinities for the methyl donor. The ribose methyltransferase has a K_m approximately 20-fold less than the 7-methylguanosine methyltransferase activity. As expected, the latter activity was inhibited first at lower concentrations of SAH than the former one (Fig. 4). These results indicate that two methylating activities are present in purified virions of VSV. Since the VSV structural proteins L and NS are known to be involved in the transcription process in vitro using the N protein-RNA nucleocapsid as the template (14, 20), it is at present unclear whether the methyltransferases are part of these proteins or separate proteins of nonviral origin.

One approach to this question was to purify

VSV from different host cells and assay the two methyltransferase activities. It is clear from the experiments shown in Fig. 6 that, irrespective of the source of the host cells, such as BHK (hamster), L (mouse), or HeLa (human), the two methyltransferases are present in the purified VSV with approximately similar activities. These results indicate that if the methyltransferases are of cellular origin, then they are present in three different species of host cells or, on the other hand, they may be encoded by the virus genome. We feel these activities may be virus specific because: (i) the methylase activities are associated with purified VSV cores (1), which contain few detectable contaminant proteins; (ii) NDV, a cytoplasmic virus, does not contain the 2'-*O*-methyltransferase activity when purified from chicken embryo fibroblast cells whereas VSV, also a cytoplasmic virus, contains both of these activities when purified from the same host cells (R. J. Colonno and H. O. Stone, personal communication); (iii) the methyltransferases present in vaccinia virus and reovirus have different characteristic properties than the VSV methyltransferases, although they too have been grown in the same host cell lines, i.e., HeLa and L cells, respectively (18, 22). Further experiments along this line will conclusively establish the origin of these methyltransferases.

Finally, the methyltransferases in the VSV system have allowed us to obtain VSV mRNA species containing the novel 5'-blocked structure—G(5')ppp(5')A_p^m.... This type of blocked and methylated 5'-terminal structure has not previously been reported in any eukaryotic mRNA species. This finding prompted us to investigate the specific effect of the 2'-*O*-methylation in the capped structure of mRNA's in in vitro protein synthesis since it has been shown previously that ^{7}mG plays an important role in recognition of mRNA's to ribosomes during initiation of protein synthesis (8). Results shown in Fig. 7 clearly show that, in wheat germ extracts, mRNA's containing the Cap B structure are identical to the unmethylated mRNA's in that they fail to incorporate amino acids into protein, whereas the dimethylated mRNA's, i.e., Cap A structure, act as excellent templates for protein synthesis. This demonstrates that mRNA's containing only the ribose methylation in the 5'-terminal cap are not sufficient to direct protein synthesis in wheat germ extracts. Nevertheless, when direct binding of the mRNA's with 80S ribosome complex was studied (data not shown) it was observed that Cap B-containing mRNA's bind consistently higher than their unmethylated counterparts similar to that observed previously using synthetic ribopolymers (26). The precise nature of binding of the Cap B-containing mRNA's to ribosomes is under investigation.

ACKNOWLEDGMENTS

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